

Controlling Aflatoxin and Fumonisin in Maize by Crop Management

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ABSTRACT

Maize is a vital food and feed grain worldwide. Aflatoxin and fumonisin, mycotoxins produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* Speare, and *Fusarium moniliforme* J. Sheld, respectively, are very potent carcinogens in both humans and livestock and can readily contaminate maize grain in the field and in storage. Stress on developing maize, particularly during reproductive growth, facilitates infection by the fungi, production of mycotoxins and contamination of the grain. Drought, excessive heat, inadequate plant nutrition, insect feeding on developing kernels, weeds, excessive plant populations, and other plant diseases can produce plant stress and facilitate the infection of maize grain by mycotoxin producing fungi. Timely planting of adapted hybrids, proper plant nutrition, irrigation, and insect control either by insecticides or the use of transgenic hybrids all assist in curbing mycotoxin contamination. Production practices that produce high yields

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are basically the same ones that help control mycotoxins. Care must also be exercised in harvesting and handling grain in transport and storage to reduce kernel breakage and prevent contamination. Harvesting early and artificial drying helps reduce the incidence of mycotoxins as well as preventing kernel breakage and stored-grain insect infestations.

Key Words: Aflatoxin; Fumonisin; *Bacillus thuringiensis* beliner; *Aspergillus flavus*; *Aspergillus parasiticus* speare; *Fusarium moniliforme* J. Sheld; Irrigation; N-Fertility; Plant stress.

INTRODUCTION

Maize (*Zea mays* L.) is a vital feed and food grain worldwide. It responds well to irrigation and fertilizer to produce a large amount of consumable calories ha⁻¹. Being a cross-pollinated crop, relatively easy to genetically manipulate, great advances have been made during the last half-century in improving the plant's architecture, pest resistance and overall yield characteristics. As of 1993, global demand for maize grain stood at approximately 526,000,000 t and is expected to rise to 784,000,000 t by 2020 (Rosengrant et al., 1999). A significant amount of that increase is expected to come from developing countries. Improvements in the living standards of people in less developed countries will likely result in dietary shifts that include more red meat and poultry in their daily food intake. The principle source of feed grain for the livestock needed to meet this demand will be maize.

To meet the expected increased demand for maize grain, the annual yield increase will need to be approximately 1.5% per year. This excludes any possible increase in land area placed into maize production in certain parts of the world. In the United States, the current rate of increase in yield is about 1.0% per year, with most of this increase being a result of improved yields per unit of land area (Duvick and Cassman, 1999). The United States, more so than any other developed country, has lost considerable amounts of prime agricultural land, where maize was previously produced, to urbanization. Housing developments and their corresponding shopping areas permanently remove land from any potential food and feed production. Population increases, not only in the United States, but worldwide will exacerbate this problem in the foreseeable future.

OVERVIEW OF MYCOTOXINS IN MAIZE

Maize grain, like other cereals, is subject to destruction by a number of pests, both in the field prior to harvest and during storage. Included among

these pests are several fungal species than can render the grain unfit for human or livestock consumption. The problem lies not with any off-flavors or loss of nutritional value the fungi may cause, but with secondary metabolites several species are capable of producing which are extremely toxic to warm blooded animals and man. These mycotoxins, as they are referred to, are capable of causing sudden death in poultry and livestock when concentrations are high. At lower levels in feed they can cause animals to become unthrifty, gaining at slow rates or making no weight gains at all (Cheeke and Shull, 1985). In dairy animals, certain mycotoxins can be passed through the animal into the milk and become a human health hazard. At low levels in food, they are known to be responsible for the onset of terminal illnesses such as liver and esophageal cancer. The U.S. Food and Drug Administration has set a limit of 20 ng g^{-1} aflatoxin contamination on maize grain that can be marketed (Park and Liang, 1993; Payne, 1992; U.S. Food and Drug Administration, 2001). Maize grain exceeding these levels cannot enter inter-state commerce and can only be used for livestock feed. Limits have also been set with respect to this use and vary with the species of livestock being fed (Table 1).

The two most common and toxic mycotoxin compounds are aflatoxin, produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* Speare, and fumonisin produced by *Fusarium moniliforme* J. Sheld. Aflatoxin is associated with human liver cancer. Aflatoxin B1 has been classified as a probable human carcinogen by IARC (1987). Fumonisin are known to cause leukoencephalomalacia in horses and pulmonary edema in swine (Harrison et al., 1990; Kellerman et al., 1990). Medical and veterinary science is continuing to learn more about other possible diseases these mycotoxins may cause.

In the Corn Belt of the United States *Aspergillus flavus*, *Fusarium moniliforme* and their corresponding mycotoxins are considered primarily

Table 1. Maximum allowable levels (action levels) of aflatoxin in maize grain.^a

Grain use	Action level (ppb)
Feed for finishing cattle	300
Feed for finishing swine	200
Feed for breeding stock (cattle & swine) and mature poultry	100
Feed for immature livestock	20
Feed for dairy animals	20
Food maize grain	20

^aFrom: U.S. Food and Drug Administration (2000).



storage problems, although field infection by *A. flavus* and aflatoxin contamination prior to harvest have been documented (Lillehoj et al., 1978; Qasem and Christensen, 1960; Rambo et al., 1974). Drought and high ambient temperatures during kernel filling have been identified as the environmental conditions most conducive to aflatoxin contamination in maize (Lisker and Lillehoj, 1991; Vincelli et al., 1995). Such environmental conditions are less common in the Corn Belt than in the southeastern United States. Considerable attention is therefore given in the Corn Belt to bin sanitation and artificially drying grain to levels safe for long-term storage. Drought and heat stress are more common during the time that maize matures in the southeastern United States. As a result, aflatoxin contamination of maize grain prior to harvest is considered more of a problem in that region of the country than elsewhere in the United States.

Other regions of the world, such as Latin America, Africa and southern Asia have experienced more problems with mycotoxin poisoning than the United States. This is likely due to limits on financial resources, infrastructure and machinery needed to manage maize production to comparable levels as American farmers. Fertilizer, especially nitrogen sources, irrigation water, harvesting, transportation, handling and storage facilities are often not as available or substantial as what is found in the United States. Limited financial resources impede the purchase and use of hybrid cultivars. As a result maize cultivars grown in these countries are often open-pollinated types or blends, which lack the heterosis of hybrids (Hallauer et al., 1988). As a result they do not have the genetic propensity for tolerating drought stress as well as most of the hybrid cultivars grown in the United States and are thus more susceptible to fungal infection and aflatoxin or fumonisin contamination. Research by Zuber et al. (1983), reported that open pollinated cultivars of maize commonly grown in the southeastern United States prior to extensive use of hybrids, were more susceptible to preharvest aflatoxin contamination than were hybrids.

Sound crop management practices have been found to be one of the more effective ways of avoiding or at least diminishing *A. flavus* and *F. moniliforme* infection and subsequent mycotoxin production. Work is currently underway at several federal, state and private facilities to develop germplasm resistant to field infection by these fungi. Genetically controlled chemical and physical methods of resistance to *A. flavus* have been researched and identified. Lozovaya (1998), suggested the enzyme β -1-3-Glucanase, when present in maize kernels, may have a role in the inhibition of *A. flavus* growth on the grain. Huang et al. (1997) has identified two proteins in kernels of the resistant maize inbred Tex 6. One protein, with a mass greater than 100 kDa inhibits aflatoxin production with no effect on fungal growth. The other protein, with a mass of 28 kDa inhibits the growth of *A. flavus*. Chen et al.

(1998), found a 14 kDa trypsin inhibitor in high concentrations in maize genotypes that were resistant to both *A. flavus* and *A. parasiticus* and at low concentrations in susceptible genotypes.

Other types of resistance include kernel pericarp wax and husk covering over the ear. Guo et al. (1995) concluded that wax and cutin layers on maize kernels may play a role in resistance to aflatoxin accumulation in certain genotypes. Russin et al. (1997) using a known resistant genotype and several susceptible commercial hybrids showed that the resistant genotype had an abundant amount of wax deposits on the kernel surfaces while the susceptible hybrids did not. Some of the early research on resistance to *A. flavus* infection and aflatoxin contamination involved the indirect protection of developing kernels by long tight husk coverings which helps reduce the feeding of insects that aid in the infection process (McMillian et al., 1985; Lisker and Lillehoj, 1991).

Despite these discoveries, little of this genetic material is being publicly advertised as being mycotoxin resistant. Its effectiveness may be limited by environmental conditions in the field, which needs to be thoroughly researched. In all probability, any genetically resistant material will require good crop management practices be employed in growing the crop for full resistance to be realized and mycotoxin contamination prevented. Even if field infection and contamination is prevented or curtailed, sound management practices during harvest, handling and storage will continue to be needed to avoid losses due to mycotoxins.

The management practices that have been found effective at reducing the incidence of mycotoxin contamination in the field include timely planting, proper plant nutrition, especially adequate amounts of N, avoiding drought stress, particularly during kernel filling, controlling certain insect pests and proper harvesting (Anderson et al., 1975; Jones and Duncan, 1981; Lillehoj, 1983; Lisker and Lillehoj, 1991). Generally crop production practices that produce high maize grain yields have been the same that coincide with significant reductions in mycotoxin contamination. Surveys conducted in North Carolina in 1977 and 1978 on maize grain yields and aflatoxin contamination revealed a negative correlation between yield and levels of preharvest aflatoxin contamination (Duncan, 1979).

EFFECTS OF PLANTING DATE ON MYCOTOXINS IN MAIZE

Research in North Carolina demonstrated that lower levels of aflatoxin B1 contamination occurred in maize grain produced by April plantings as compared to May plantings (Jones and Duncan, 1981; Jones et al., 1981).



Lillehoj et al. (1978) also report that a higher incidence of aflatoxin B1 was observed in June-planted maize compared to April and May plantings in 1976 from experiments conducted in Florida and Georgia. Zuber and Lillehoj (1979) surmised that early planting shifts the period between anthesis and dough-development in maize to a time frame in the growing season where drought and heat stress are less likely to be encountered, as compared to later plantings. However, Widstrom et al. (1990) discovered that in the Coastal Plain of Georgia early April plantings of maize are at greater risk to aflatoxin contamination than are plantings made at mid-May or later. They concluded that early plantings were at higher risk because the critical period in kernel filling that begins approximately 20 d after anthesis occurs when seasonal maximum and minimum temperatures as well as net evaporation are highest. These environmental conditions are most favorable to *A. flavus* infection and aflatoxin production. A survey in this same region conducted in 1978 found grain samples taken in September had lower levels of aflatoxin than those taken in July (McMillian et al., 1980).

Payne (1999) reported that the optimum conditions required for fumonisin production are not known. However, the occurrence of *F. moniliforme* appears to be related to drought stress, particularly early in the growing season which results in an increase in infection and systemic colonization of the fungus. A later U.S. FDA report (U.S. Food and Drug Administration, 2000), states that fumonisin levels in raw maize grain are favored by environmental conditions of heat and drought stress followed by periods of high humidity. As with aflatoxin, fumonisin appears to be more prevalent in the southern United States than elsewhere in the country. Shelby et al. (1994) found fumonisin levels in maize grain of several hybrids increased as their production occurred further south. They also observed that hybrids with high levels of aflatoxin contamination in earlier observations were also high in fumonisin.

MOISTURE AND HEAT STRESS ON THE INCIDENCE OF MYCOTOXINS

Drought and high ambient temperatures appear to favor the production of these fungi and their corresponding mycotoxins. Lillehoj (1983) observed a connection between drought stress and the presence of aflatoxin. Manwiller and Fortnum (1979) estimated that approximately 90% of the maize grown for grain in South Carolina in 1977 and 1978 was contaminated with aflatoxin due to drought and heat stress. Vincelli and Parker (2001) concluded that fumonisin contamination of maize grain is favored by drought stress before and during anthesis. Fumonisin concentrations

have been reported to be inversely related to late spring and early summer rainfall (Shelby et al., 1994).

Irrigation has been reported to reduce *A. flavus* infection and aflatoxin concentration in maize (Jones et al., 1981). It was also observed that a greater contrast in these differences occurred during a year of lower than normal rainfall. However, Stoloff and Lillehoj (1984) observed higher concentrations of aflatoxin in maize produced in 1980 than in 1979 despite the application of irrigation. They concluded that preconditioning maize for production of aflatoxin probably involves several factors that work independently or interactively to alter aflatoxin levels in the grain at harvest. The impact irrigation has on the incidence of fumonisin is not well documented. However, Miller (2001) concluded that the best available strategies for reducing the incidence of fumonisin in maize are to plant hybrids adapted to the environment, limit drought stress and minimize insect feeding.

Fortnum (1986) stated that in the southeastern United States high temperature may be the most important environmental factor influencing preharvest infection of maize by *A. flavus* and the subsequent production of aflatoxin. Jones et al. (1980) observed that high ambient temperatures favored the infection of maize by *A. flavus* and aflatoxin development in the grain before harvest. However, Setamou et al. (1997) reported that *A. flavus* infection and aflatoxin contamination observed in maize produced in Benin were complex phenomena and were mediated by more than temperature alone.

Several researchers report the need for planting maize early in the growing season to avoid or reduce the risk of drought and heat stress conducive to the mycotoxin problem. However, it appears the key is to time the onset of reproductive growth in maize to environmental conditions that are less stressful to the plant and less favorable to fungal development regardless of the time of the year. It appears necessary to determine optimum maize planting dates for each production area. Climatic conditions need to be scrutinized to match the maize-growing season with the environment that is least likely to facilitate fungal infection. These will likely be the conditions that also produce the highest grain yields.

PLANT NUTRITION AND MYCOTOXIN INCIDENCE

Adequate plant nutrition, particularly sufficient levels of nitrogen (N), are known to be important in reducing the risks of fungal infection and the development of mycotoxins (Jones, 1979; Lillehoj and Zuber, 1975). Nitrogen is the central element in structural and metabolic proteins as well as nucleic acids. Maize plants suffering from N deficiencies during reproductive growth, will often translocate N from older leaf tissue to the



developing grain and eventually abort the older leaves. Plant stress resulting from low N-fertilization rates was found to increase the incidence of aflatoxin contamination in maize (Lillehoj and Zuber, 1975). Jones (1979) stated that maize might be predisposed to aflatoxin contamination due to insufficient uptake of nutrients associated with drought stress or leaching of mineralized N from the root zone due to excessive rain. A research team from Quaker Oats reported higher levels of aflatoxin contamination in maize fertilized with 60 units of N fertilizer compared to maize fertilized with 140 units (Anderson et al., 1975). Jones and Duncan (1981) reported that maize grown with low levels of N-fertility (11.2 kg ha^{-1}) had consistently higher aflatoxin levels than grain produced with high N-fertility rates (145.7 kg ha^{-1}). When the results were averaged among planting dates, isolates and cultivars, the low N-fertility treatments were observed to have 2.4 times more aflatoxin B1 than the high N-fertility treatments.

Research on the effects of other plant macro-nutrients and the incidence of mycotoxins in maize is currently unavailable or very limited. Basically, an inadequacy of any nutrient element increases a plant's susceptibility to attack by most all forms of plant pathogens (Stromberg et al., 1999). Visible deficiency symptoms represent the most severe form of negative expression in plant metabolism and development. A phosphorus (P) deficiency in maize during the early weeks of growth can result in a poorly developed root system, which in turn can reduce the plant's ability to take up adequate levels of other essential nutrients and water (Stoloff and Lillehoj, 1984). This could logically lead to the early onset of drought stress, which has already been discussed as a prerequisite to fungal infection and mycotoxin development. Phosphorus is also important to plant growth as it is incorporated into a number of vital biological compounds. It is a key element in nucleotides by virtue of being a component of the phosphate sugars found in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Bruns, 1991). It is also the key component in energy transfer compounds such as adenosine tri-phosphate (ATP). This compound serves a number of vital functions, one of which is facilitating peptide bonds between certain amino acids in the formation of proteins. Development of maize genetically resistant to mycotoxins will involve nucleotides and synthesis of proteins that will impart the resistance. Sufficient levels of P will be required for the expression of such a trait in hybrid cultivars that are developed.

Potassium (K) is found in large quantities in plants and a number of its functions are still to be discovered. One very important role however, that may relate to fungal infection and mycotoxin production, is its regulation of cellular hydration and stomatal activity. Due to its size and mobility, K acts as a moisturizing agent to maintain hydration of cell organelles, including semi-permeable membranes (Shelby et al., 1994). Leaf stomata, which are

important in gas exchange, moderating plant temperature and regulating the water potential of the plant are controlled by varying concentrations of K in the guard cells and adjacent leaf cells. Proper function of the stomata is essential to photosynthesis and subsequent plant growth. As with P, deficiencies of K could result in the early onset of drought stress and subsequent fungal attack.

Calcium (Ca) has an important role in root growth and development of plants. A major role and one that is well documented is to counteract the deleterious effects of low pH on ion availability in the soil solution and nutrient uptake in general (Epstein, 1972). Some nutrients, such as P, become less available with increased soil acidity and can become deficient in extreme conditions. Still others become more available to the point of being toxic to plant growth. It is believed Ca serves to render innocuous, levels of other cations that are toxic to plant growth (Epstein, 1972). For example in one study, cotton (*Gossypium hirsutum* L.) roots failed to grow in a Ca free soil (Rios and Pearson, 1964). Calcium is also important in the construction of plant cell walls and a deficiency later in the growing season will adversely affect the growing points of a plant, particularly those of the roots (Epstein, 1972). Two points can be logically assumed from this information. First, a lack of sufficient Ca can impair maize root growth and thus impede the uptake of sufficient water to prevent drought stress. This would also negatively affect the uptake of other essential nutrients and exacerbate the level of stress experienced by the plant. Second, the impairment of cell wall development may well make developing maize grain more subject to fungal attack and subsequent mycotoxin contamination. Blossom end rot in tomato (*Lycopersicon esculentum* Mill.) is known to be linked to Ca deficiency in developing fruit and attack by fungi via weakening cell walls (Hodges and Steinegger, 1991).

WEEDS AND MYCOTOXINS IN MAIZE

Weeds are not known to directly cause the infection of maize by *A. flavus* or *F. moniliforme* but their deleterious effects on crop yields are very well documented. With regard to stress upon developing maize, weeds rob the crop of water, nutrients and sunlight. Certain weed species are also known to exude chemicals, via their roots into the soil, that stunt crop development, a process known as allelopathy (Rice, 1984). Heavy weed infestations in maize place the crop under considerable stress due to the competition they create. Maize crops harvested from weed-infested fields suffer yield reductions and decreased grain quality similar to those harvested from drought stressed fields (Rice, 1984). Lillehoj (1983) stated



that a weed canopy in maize contributes to the contamination of grain by aflatoxin due to the stress they exert on the crop. Earlier Cobb (1979) had linked the presence of aflatoxin in maize to the competition for essential growth substances imposed by the presence of weeds and the stress they created on the crop.

EFFECTS OF INSECTS ON PRE-HARVEST MYCOTOXIN CONTAMINATION

Insect feeding activity has been found to be associated with fungal infection of maize grain and the subsequent production of mycotoxins (Beti et al., 1995; Drepper and Renfro, 1990; Lillehoj et al., 1975; Sauer and Burroughs, 1980). Both *A. flavus* and *F. moniliforme* are known to be facilitated in their infection process of maize grain by insect feeding (Beti et al., 1995; Drepper and Renfro, 1990). Setamou et al. (1997) reported maize ears with less than 2% insect feeding damage had a mean aflatoxin contamination level considerably lower in both 1994 and 1995 than ears with more than 10% damage. It was reported nearly a century ago that the incidence of moldy ears of maize increased in years with high populations of insects (Garman and Jewett, 1914). A survey conducted in southeast Missouri and southern Illinois by a team of USDA-ARS scientists in 1972, concluded that maize ears that had been extensively damaged by European corn borer [*Ostrinia nubilalis* (Hubner)] and corn earworm [*Heliothis zea* (Boddie)] had significantly higher levels of aflatoxin than undamaged ears (Lillehoj et al., 1975). There was also an indication that insect vectoring of the fungal inoculum occurred due to larvae ingesting spores and transferring the infection to developing kernels through their frass. In experiments conducted in Georgia in 1974, Widstrom et al. (1976), found a relative increase in corn earworm damage of maize grain produced from 2 May plantings compared to 19 April plantings. Research by Windels et al. (1976) determined that picnic beetles (*Glischrochilus quadrisignatus*) carried *Fusarium* spp. spores both internally and externally. It was determined that these insects were likely vectors of the fungus to maize due to their habit of visiting developing corn ears and wounds on the ear produced by other insects. McMillian et al. (1980) found that *A. flavus* sporulation and aflatoxin contamination increased in maize damaged by corn earworm and fall armyworm (*Spodoptera frugiperda*) feeding on the developing grain.

Controlling ear feeding insects has generally been limited to high-cash value food maize such as sweet corn or white maize. Yellow maize grown for livestock feed is seldom treated with insecticides to control such pests, except in irrigated fields grown under intense management. Chemical control of European corn borer requires scouting fields to determine the

stage in the insect's lifecycle and timing the application of insecticide to obtain maximum control. Late treatment of infested fields will be ineffective once the borer enters the interior of the stalk. Corn earworms can be chemically controlled; however, numerous insecticide applications are required, making the cost prohibitive for feed grain.

The development of transgenic maize containing the gene for Bt δ -endotoxin (*Bacillus thuringiensis* Beliner) has considerably changed the scope of maize insect control. The Bt proteins are crystalline in nature and referred to as "Cry proteins." Maize hybrids containing the Cry proteins are especially helpful in the control of a number of lepidopteran insects in the larval stage (Williams et al., 1998). Maize hybrids expressing the Cry1Ab Bt protein have been observed to experience less *Fusarium* infection due to the association between insect feeding and the pathogen (Munkvold et al., 1997). Information on the effect Cry proteins may have on *Aspergillus* spp. infection and subsequent aflatoxin production in maize is unavailable. Cotty et al. (1997) reported that cottonseeds of transgenic Bt cultivars sometimes have lower levels of aflatoxin contamination. It would be logical to assume that if the presence of Cry proteins in maize results in a reduction of feeding by insects known to vector mycotoxin producing fungi, then lower levels of the mycotoxin could result in Bt hybrids as compared to non-transgenic cultivars. Research on mycotoxins and Bt maize hybrids is currently underway at several locations.

PLANT POPULATION STRESS AND MYCOTOXINS

Excessively high plant populations can stress maize crops and likely facilitate mycotoxin contamination. This was especially true when aflatoxin was first identified in the early 1960's. Open pollinated cultivars and hybrids, which were usually double-cross genotypes, grown at that time responded negatively to high plant populations with yield reductions (Mitchell, 1970). One such response would be an increase in barren plants, plants without an ear or a grainless ear (Manwiller and Fortnum, 1979). Cultivars selected to be grown at plant populations of 30,000 to 40,000 plants ha⁻¹ would often respond to plant densities of 49,000 to 59,000 plants ha⁻¹ with barren plants. Modern maize hybrids, which are mostly single-cross genotypes, have been developed genetically to withstand plant-to-plant competition better than some of the hybrids grown as late as the 1980's. Plant population recommendations for maize production have steadily increased to where in most cases; they are now double those of the 1950's. One method of improving the maize plant's ability to withstand population increases is to change the plant's architecture to include an erect up-right leaf. This allows greater penetration of light into the crop canopy and reduces shading which is believed to have



reduced silk length in older plant types (Mitchell, 1970). Improvements have also been made in disease and insect resistance, which enhances a maize crop's ability to produce under high plant populations. There are limits however. High plant populations require lots of water and fertility, especially N. Irrigation is essentially a requirement under these production conditions, particularly in the Mid South and Southeastern United States. Maize crops not supplied with sufficient amounts of these essentials throughout the growing season will become stressed very quickly and severely, thus becoming subject to fungal infection and mycotoxin production. Good management practices dictate close monitoring of maize being produced at a high plant population to avoid stress and mycotoxin problems.

OTHER PLANT PATHOGENS AND MYCOTOXINS IN MAIZE

Fungi that cause ear rots such as those caused by *Aspergillus* spp. and *Fusarium* spp. are opportunists, infecting and contaminating maize grain with mycotoxins after the plants have been stressed by some other factor, including other plant diseases. Root rot diseases are considered a disease complex involving a number of different fungi, nematodes, root-feeding insects and even some bacteria (White, 1999). Foliar diseases, such as blights and rusts, are also serious pests of maize and can greatly weaken the plant. Stalk rots, which are caused by a number of different pathogens may result in the premature death of the maize plant just before the developing kernels have reached physiological maturity. All such diseases are referred to as primary pathogens. Any of these events increase the chances for infection of the grain by mycotoxin producing fungi, which are referred to as secondary pathogens or infections.

The methods most commonly employed in combating primary plant pathogens in maize are the use of adapted disease resistant cultivars, when available, and cultural practices (Mitchell, 1970). Pesticides for control of diseases in maize grown for feed are seldom used because the cost of chemical control is often prohibitive. Hybrid maize is usually bred to fit a narrower environmental range compared to most other crop species. All successful hybrid maize seed companies usually have a group of hybrids that can be successfully grown in a particular environment. They will also tailor a number of their hybrids to be resistant to the diseases commonly found in a region. Genetic resistance to various rusts, gray leaf spot (*Cercospora zeae-maydis*), and Southern leaf blight [*Cochliobolus heterostrophus* (Drechs.)] are examples of maize diseases that are controlled largely through the use of host plant genetic resistance (White, 1999).

Cultural practices primarily involve clean tillage and crop rotation. Each of these procedures has long been considered standard methods for plant disease control (Mitchell, 1970). Minimum tillage and no-till maize production however, have changed thinking on the subject. Minimum and no-till crop production in general, have benefits that conventional farming procedures do not have. The principle advantage of these two cropping methods is a reduction in soil erosion and the problems associated with it. Another advantage of these cropping practices is a reduction in the number of trips made across a field during a growing season. Not only is the consumption of fuel by machinery decreased, but it helps reduce soil compaction as well. Residue from the previous year's crop is left on the soil surface as mulch and aids in water conservation. It is believed that mulch helps reduce the evaporation of moisture from the soil and in turn reduces the incidence of stalk rot diseases. However, this residue can be a source of inoculum for foliar diseases, which can infect and weaken the plants making them susceptible to the mycotoxin producing fungi. It is also believed to increase the incidence of *A. flavus* infection of the grain (Streeter and Barta, 1984).

Crop rotation has a number of advantages beyond aiding in the control of crop diseases. Improvements in soil structure, water conservation, reduced soil erosion and decreased levels of allelochemicals have all been identified as benefits to crop rotation (Olson and Sander, 1988). The larger benefit, however, is a maintenance or improvement of maize yield levels. Prior to the extensive use of agricultural chemicals, crop rotation was the primary means of pest control. Introducing a less favorable host crop periodically inhibited or greatly reduced the population and/or incidence of certain pests. Adams et al. (1970) observed improvements in maize yields following sod and annual green manure crops, which exceeded those of continuous maize that had 180 kg ha^{-1} fertilizer N applied to the soil. On plots that had previously been in sod the benefits from the sod crop on soil tilth and a reduction in nematode populations were found to persist for 3 to 4 years. Allelochemical effects are not completely understood but crop rotation is known to reduce or remove the effect of root excretions and their subsequent biological effect upon crop species grown in a monoculture (Olson and Sander, 1988; Rice, 1984).

HARVESTING, HANDLING, AND STORAGE TO REDUCE POST-HARVEST CONTAMINATION

Harvesting techniques and methods can have an influence upon the incidence of mycotoxin contamination in maize. The general recommendation



is to harvest maize grain at a moisture content of between 255 to 200 mg g⁻¹ and then artificially dry it to 155 mg g⁻¹ for safe storage (U.S. Food and Drug Administration, 2000). Fungal growth and mycotoxin production can flourish in a matter of days if maize grain is not properly dried and cooled before being placed in storage (Setamou et al., 1997). Even during transportation it is important that grain be at a safe moisture and temperature if it is going to be in route over several days.

In much of the world including areas of the Mid South and southeastern United States, grain-drying facilities are few in number and as a result many maize growers depend on field drying to obtain a grain moisture level safe for handling and storage. The time required to reach a level of 155 mg g⁻¹ grain moisture content depends upon the weather but will generally be from 14–28 d post physiological maturity (Bruns and Abbas, 2001). Maize growers using this method of drying need to monitor fields closely so as not to let the grain over-dry. Maize grain drier than 155 mg g⁻¹ is very subject to kernel breakage during harvesting (Vincelli and Parker, 2001). Broken kernels are especially subject to fungal infection as the surface of the kernel's germ is exposed. Research has shown that damage to the germ greatly increases development of grain storage molds in maize (Seitz et al., 1982; Tuite et al., 1985; Vincelli and Parker, 2001).

Properly setting combines and driving harvesting machinery at ground speeds that do not overload the threshing mechanism are effective ways to minimizing kernel breakage (Watson, 1988). Grain entering the hopper of the combine should be monitored during the harvest and adjustments to the machine made to compensate for changes in kernel moisture content, kernel size and ear size. Plentiful amounts of air should be blown through the grain during threshing to remove foreign matter, weed seed, and light chaffy grain that may be infected with fungi and contaminated with mycotoxins. Areas in the field that have been severely stressed should be avoided during harvest if it is suspected that mycotoxins may be a problem.

Other practices prior to and during harvest are also important to minimizing the risk of mycotoxin contaminated maize. Combines should be cleaned internally before harvesting begins, removing any residue from previous harvests. If a grower plans to store the grain for use on the farm or delay placing it on the market, bins need to be thoroughly cleaned on the inside. All old grain should be removed, the sides of the bin swept down and treated with an approved insecticide for control of stored grain pests. Old grain on the outside of the bin should be removed as well as this too can harbor stored grain insect pests that could infest new grain and eventually lead to the growth of mycotoxin forming fungi. Areas within a bin of grain that become infested with stored-grain insects are referred to as hot spots. The increase in insect populations and their metabolic activity

results in elevated grain moisture levels and fungal growth (Mills, 1983). On farms, new grain should never be placed on top of old. Differences in moisture content of the old and new grain, insect levels or the presence of fungi can result in a loss of the entire bin of grain in a very short period of time. Grain of different moisture contents is frequently blended at elevators or other large grain storage facilities so as to reduce the risk of spoilage. However, great care is required in the blending operation to prevent spoilage. Sauer and Burroughs (1980) found that blended corn samples with moisture contents of 177 mg g^{-1} and stored in an equilibrium relative humidity of 86–87% became heavily infested with *A. flavus* and contaminated with aflatoxin within a short period of time. Samples that were stored at an equilibrium relative humidity of less than 85% had a limited amount of aflatoxin contamination after the same period of time. Artificially drying maize grain before storage or shipping can be a wise investment as it will reduce the continued growth of fungi and basically halt mycotoxin production. However, grain drying is not a matter of just applying heated air to a bin full of maize until it reaches a certain moisture level. Improper attempts to artificially dry maize can actually worsen the situation and result in more rapid spoilage or destroy the grain if it is overheated. Individuals planning to purchase on-farm drying equipment would be well advised to get formal instruction on the equipment's use.

CONCLUSIONS

Mycotoxins in maize result from the plant experiencing stress of any type during its reproductive growth. Genetically resistant hybrid cultivars of maize may eventually be available on a large scale, but in all likelihood will require sound production management and storage practices in order to be completely effective. Production of maize with mycotoxin levels below the maximum allowable levels for commerce is very dependant upon avoiding high temperatures and high humidity during reproductive growth. What constitutes the best planting time, optimum fertility levels and the best adapted cultivars appears to be regional in nature and will need to be continually researched in the specific areas where maize will be produced. Adequate soil moisture, either through rainfall, irrigation or a combination of both is vital to avoiding problems with aflatoxin and/or fumonisin regardless of the region. The use of transgenic maize, especially for insect control, appears to hold promise for reducing the incidence of mycotoxins as well by controlling those pest that have been shown responsible for vectoring fungal infections. However, continued research into newer transgenic hybrids with different Bt events will be needed to replace current hybrids that may lose effectiveness



in controlling insects over time. Care in harvesting, handling and storing maize grain to prevent post-harvest fungal infection and mycotoxin contamination is as vital as properly managing the growth of the crop to prevent pre-harvest infection. Educating growers, handlers and marketers about grain handling methods to reduce post-harvest contamination is important to the over-all objective of providing safe food and feed grain.

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